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FUNGAL ALKALINE PROTEASES: APPLICATIONS AND PROPERTIES

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ABSTRACT

Synthetic chemical usage enhanced day by day at both industrial level and medical fields, together causing vulnerability in the ecological pyramids and leads to loss of ecological equilibrium around the globe. In addition, the enormous growth of industries, affecting the health of both environment and community health. For the betterment of life to achieve in the community, step has taken to develop the different processes for eco-friendly products. Instead of chemical usage, enzymatic processes are the alternatives to attain the healthy environment. Take a glance on the Alkaline proteases derived from fungi having various industrial applications and eco-friendly in nature, includes textile, detergent, leather, feed, waste, water recycling, bioremediation, tannery effluent, effluent treatment and medical applications. The present discussion focused on alkaline proteases applications and properties. Type of production with various influencing parameters.
Interpreting the results and depicting the multifunctional properties used to promote fungal alkaline proteases became versatile in the fields of eco-friendly and medicinal applications, which has gained prominent usage of stable properties.

KEYWORDS: Fungal proteases, proteolytic enzymes, alkaline enzymes, physicochemical properties of enzymes, substrate-specific, applications of proteases.

INTRODUCTION:

Enzymes are highly specific and act under mild conditions than traditional chemicals (Elaine, Luo (2018)). Readily biodegradable and usually no toxic to the environment, dumped from the industries (Layla Filiciotto and Gadi Rothenberg 2020).

Industries produces many types of enzymes by using the various production processes, such as pulp and paper production (Industrial Uses Of Enzymes, Infinita Biotech, (2019); Jiménez et al., 1999; Nguyen et al., 2008), leather production (Dayanandan et al., 2003; Saravanabhavan et al., 2004; Valeika et al., 2009; Kandasamy et al., 2012; Vishnuvardhan Reddy and Sultanpuram, 2016), textile production (Aly et al., 2004; Vankar et al., 2007; Chen et al., 2007; Zhou et al., 2008), detergent production (Hemachander and Puvanakrishnan, 2000; Saeki et al., 2007), food production (Minussi et al., 2002; Ramos and Malcata, 2011), beverage production (Grassin and Fauquembergue, 1996; Okamura-Matsui et al., 2003), animal feed production (Gado et al., 2009; Zhu et al., 2011; Dalel Daassi et al., 2016), pharmaceuticals production (Bonrath et al., 2002; Woodley, 2008),fine chemicals production (Panke et al., 2004; Gavrilescu and Chisti, 2005), cosmetics production (Sim et al., 2000; Lods et al., 2001) and biodiesel production (Kumari et al., 2007; Hernandez-Martín and Otero, 2008).

Enzyme-catalysed processes are gradually replacing chemical processes in many areas of industry (Peter, Robinson (2015)). Enzymes have all the properties of true catalysts (Iva Colter (2020)). In the presence of an appropriate enzyme, a chemical reaction occurs at a much higher rate but the enzyme is not consumed by the reaction (Ganapati, Yadav and Deepali Magadum (2017)).

Recently, a greater awareness of conservation issues have forced industries with a history of polluting to consider alternative, cleaner methods so there is now significant growth of biotechnology outside of the pharmaceutical and food industries (Robert et al., 2020). Serine proteases are used in various industrial sectors such as food, pharmaceutical, leather, diagnostics and waste management (Gupta et al., 2002; Maurer 2004; Razzaq, Abdul et al., 2019).

At present, only about 20 enzymes are produced on a truly industrial scale. 35 producers make these enzymes with 4 major companies holding 75% of the market (Mayuri Sharma et al., 2019).

In 1992, the market value of these enzymes was approximately US$800 million with a predicted yearly increase of around 10- 15%. The market is highly competitive, suffers from over-capacity, has small profit margins and is technologically intensive. Matching an enzyme with a process is the greatest challenge to a research and its development cost in an industrial plant can be modified to accommodate the limitations of an enzyme but this is costly and a better approach is to find an enzyme more suited to the existing process (Gallezot, Pierre. (2008)).

Worldwide Status of Proteases:

Proteases termed as Industrial masters occupy the third largest position in enzyme production .Real Hernandez and Elvira Gonzalez de Mejia (2019); Swapna Vadlamani et al., 2012).

Proteases are the most important industrial enzymes accounting for approximately 40% of the total industrial enzyme market (Gupta et al., 2002; Alya Sellami-Kamoun et al., 2006; Anderson F. Santos et al., (2013)) Proteases and alpha amylases are the two most important industrial enzymes, together representing more than 70% of the total worldwide enzyme market (Ramachandran, et al., 2004; Hamid Mukhtar and Ikram-ul-Haq, 2008; Aline Machado de Castro et al., 2018).

It is reported that in the year 2005, the global proteolytic enzyme demand for protease was increased dramatically to 1–1.2 billion dollars (Neetu, Jabalia., Mishra, and Chaudhary, Nidhee. P.C. (2014);Godfrey and West, 1996; Vida Maghsoodi et al., 2014).

Microbial protease (Proteolytic enzymes):

Constitutes one of the most valuable groups of industrial enzymes that cater to the requirements of nearly 60% of the world enzyme market (Rao et al., 1998; Woods et al., 2001; Ramnani et al., 2005; Hamid Mukhtar and Ikram-Ul-Haq, 2009; Nirmal et al., 2011; Zanphorlina, et al.,2011; Nilgun Tekin1 et al., 2012; Jayasree et al., 2013; Nai-Wan Hsiao et al., 2014; Vida Maghsoodi et al., 2014); Vida Maghsoodi et al., 2014).

Among the various proteases, bacterial proteases are the most significant (Gupta et al., 2002; Shivasharana et al., 2012) and accounts for nearly 60% of the total worldwide enzyme sales (Adinarayana et al., 2003; Beg et al., 2003; Shivasharana et al., 2012).

According to Johnvesly et al., 2002, 2/3 of the total amount of the enzymes sale in the world is of microbial origin (Johnvesly et al., 2002). 5 billion in 2000 (Kirket al., 2002). As per the forecast, the global demand for enzymes will rise 7% per annum through 2006 to $6 billion in 2011 (McCoy, 2000).

Proteases, also referred to as peptidases or proteinases, are one of the most useful, large category of degradative enzymes that catalyze the hydrolysis of peptide bonds into peptides and amino acids (Lopez- Otin and Bond, 2008; Jayasree et al., 2013; Lakshmi, et al., 2013; Nai-Wan Hsiao et al., 2014). Proteases hydrolyze protein by adding water across peptide bonds and break them in to smaller peptides in organic solvents (Al-Shehri, 2004; Charu Lata et al., 2014).

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Proteases are ubiquitous, being found in a wide diversity of sources such as plants, animals, and microorganisms (Alnahdi, 2012; Charu Lata et al., 2014). Proteolytic enzymes are high temperature resistant with high specific activities, execute a wide variety of functions and superior physical and chemical characteristics which seem to be good for future biotechnological applications, that is why they have wide applications in a large number of industrial processes and are produced in large quantities by microorganisms through the fermentation process (Rao et al., 1998; Gupta et al.,2002; Mohan et al., 2005; Temiz et al., 2008; Hamid Mukhtar and Ikram-ul-Haq, 2008; Hamid Mukhtar et al., 2009; Zanphorlina et al., 2011; Mukesh Kumar et al., 2012).

They have diverse applications in a wide variety of industries (Kumar, Takagi 1999; De Coninck et al., 2000; Gupta et al., 2002a; Gupta et al.,2002b; Mohan et al., 2005; Rai and Mukherjee, 2009; Swapna Vadlamani et al., 2012ani et al., 2012) such as detergent, food, baking, brewing, meat tenderization, cosmetics, peptide synthesis, waste treatment (Gupta et al., 2002; Padmavathi, 2013; Charu Lata et al., 2014), pharmaceutical, leather, silk, medicine diagnostics and therapeutic applications (Ward, 1985) the recovery of silver from used X-ray films (Cowan,1996; Kumar and Takagi, 1999; Alya Sellami-Kamoun et al., (2006) and bioremediation processes (Horikoshi (1999); Nilegaonkar et al., 2002; Kanekar et al., 2002; Denizci et al., 2004; Anwar and Saleemuddin, 1998; Gupta et al., 2002; Padmavathi, 2013).

Sources of Proteases:
Proteases are ubiquitous and physiologically one of the most important classes of enzymes that are expressed as essential constituents of all living organisms, on earth in a wide diversity of sources such as plants, animals, and microorganisms (Alnahdi, 2012) including prokaryotes (bacteria, archea), viruses, fungi (Gupta et al., 2002; Joo et al., 2003; Dubey et al., 2007; Shivakumar Srividya; Majumdar Mala, 2009; Shivasharana et al., 2012; Padmavathi, 2013).

The main sources of the enzymes were from animals (e.g. calf stomach), plants (e.g. pineapple, fig and papaya), microbes (e.g. Bacillus spp., Pseudomonas spp.) (Puri et al., 2002; Swapna Vadlamani et al., 2012; Shafee et al., 2005; Rao et al., 1998).

At least a quarter of the microorganisms such as bacteria, fungi, yeast, plant and mammalian tissues are known to produce alkaline proteases (Ellaiah et al., 2002; Prakasham et al., 2005; Alya Sellami-Kamoun et al., 2006; Mukesh Kumar et al., 2012).

Alkaline proteases: (EC.3.4.21–24, 99; EC 3.4.21-24)
The alkaline proteases are defined as the proteases that are active or work optimally in a neutral to alkaline pH range (Barett, 1994; Gupta et al., 2002; Lakshmi, et al., 2013) generally between 9.0 and 11.0, with the exception of a few higher pH values of about 12.0 and 13.0 and are used widely in washing powders and to dehair hides (Kumar and Takagi, 1999; Gupta et al., 2002; Ibrahim et al., 2007; Shampa Sen et al., 2009; Nilgun Tekin et al., 2012; Gupta et al., 2012; Sangeeta Saxena et al., 2014).

They either have a serine center (serine protease) or are of metallotype (metalloprotease) and the alkaline serine proteases are the most important group of enzymes exploited commercially (Ainsworth,1994; Fujiwara et al.,1993; Rao et al.,1998; Sangeeta Saxena et al., 2014;

In recent years, the use of alkaline proteases in a variety of industrial processes like detergents, food, leather and silk has increased remarkably (Kembhavi et al., 1993; Gessesse, 1997; Shampa Sen et al., 2009; Ponnuswamy Vijayaraghavan et al., 2013).

Enzymatic depilation has been widely accepted as a sound alternative to the chemical process (Taylor et al., 1987; Roja Rani et al., 2012; Gupta, 2017).

The vast biochemical diversity of alkaline protease of microbial origin with elevated activity and stability in the high alkaline range especially those from bacteria and fungi (Madan et al., 2002; Kalpana Devi et al., 2008; Jellouli et al., 2009; Wang et al., 2011) and the specificity of their action at alkaline pH (Ganesh Kumara and Hiroshi Takagib, 1999), emerging novel properties and their exotic catalytic nature have attracted worldwide scientific community of protein chemistry and protein engineering and applied fields (Lakshmi et al., 2013; Jayasree, et al., 2013) to exploit their physiological, bioengineering and biotechnological applications (Poldermans, 1990; Fox et al., 1991; Rao et al.,1998; Kumar and Takagi, 1999; Agarwal et al., 2004; Devi et al., 2008; Asokan and Jayanthi, 2010; Nirmal et al., 2011; Shivasharana et al., 2012; Lakshmi et al.,2013; Sangeeta Saxena et al., 2014).

The full potential of alkaline proteases in industrial processes is yet to be fully exploited (Cowan, 1996; Kumar and Takagi, 1999; Shampa Sen et al., 2009; Nirmal et al., 2011; Nilgun Tekin1 et al., 2012; Lakshmi et al., 2013; Jayasree et al., 2013).

Disadvantages of alkaline proteases:
Although enzymatic technology is very promising, it has limitations (Abdul Razzaq et al., 2019). Microbes can reproduce and increase their population in order to consume a large amount of substrate, but extracellular enzymes like alkaline protease cannot (Beti Vidmar and Maša Vodovnik (2018)). Enzymes cannot reproduce themselves, meaning that any increase in enzyme population must come from outside of the system namely, humans adding more enzymes to the system (Ayla Sant'Ana da Silva et al., 2020). It has also been shown that these alkaline proteases lose some reactivity after they interact with pollutants and could eventually become completely inactive (Xiao-lin Ao et al., 2018).

Advantages of alkaline proteases:
Among the different types of microbial proteases the most commercially important are the alkaline proteases, especially those from the fungal sources and were obtained from Numerous moulds especially belonging to the genera Aspergillus species such as Aspergillus sydowi (Danno and Yoshimura 1967), A.mellieus (Ito and Sugira 1968; Luisetti et al., 1991) and Aspergillus oryzae (Ito and Sugira 1968), A.fumigatus (Monod et al., 1991; Larcher et al., 1992; Roja Rani et al., 2012), A.flavus (Malathi and Chakraborthy 199), some strains of A. niger (Heneri et al., 1988) and A.sojae (Hayashi et al., 1967; Abdul Razzaq et al., 2019). Fungi elaborate a wider variety of enzymes than do bacteria.

For example, Aspergillus oryzae produces acid, neutral and alkaline proteases (Padmavathi, 2013). Fungal proteases can conveniently be produced in solid-state fermentation process (Chander, Mukesh. (2019)).

Proteases as Detergent additives:
The use of proteases in laundry detergents accounts for approximately 25% of the total worldwide sales of enzymes (Mecikoglu et al., 2006; Temam Abrar Hamza (2017)). Alkaline proteases useful for detergent applications were mostly active in the pH range 8 - 12 and at temperatures between 50°C-70°C (AlShehri, 2004). Many investigators (Kim et al., 2001; Charu Lata et al., 2014) studied optimal culture condition for protease productivity. Proteases produced by Bacillus spp., find a wide variety of application in detergents, leather industries, food and pharmaceutical industries (Ammar, et al., 1991).

Alkaline proteases are of special interest as they have excellent washing performance at pH (9-11), which removes protein based stains in both laundry and automatic dish washing detergents (Saeki et al., 2007; Dias et al., 2008; Mukesh Kumar et al., 2012).Bacteria (Hamid Mukhtar; Ikram-ul-Haq, 2008) mainly produce the proteases used in detergent industries. 1991; Jany and Mayer 1985; Kwon et al., 1994).A combination of lipase, amylase and cellulase is expected to enhance the performance of protease in laundry detergents (Padmavathi, 2013).

Proteases in Leather Industry:
The proteases widely used in leather industries (Kalisz, 1988; Inhs et al., 1999; Kumar and Tagaki, 1999; Ramachandranet al., 2004; Hamid Mukhtar and Ikram-ul-Haq, 2008).

The conventional methods in the leather processing industry is one of the worst offenders of the environment, due to pre-tanning operations involves quite toxic chemicals such as hydrogen sulphide creating environmental pollution, effluent disposal (Rani et al., 2012) and safety hazards (Okumura, et al., 1977; Malathi and Chakraborthy, 1991; Shivasharana et al., 2012; Padmavathi, 2013). Proteases are used for selective hydrolysis of non-collagenases constituents of the skin and for removal of nonfibrillarproteins such as albumins and globulins (Padmavathi, 2013).Of particular importance are the proteases with activity at alkaline pH and high temperatures (Kumar and Takagi, 1999; Thangam and Rajkumar, 2002; Agrawal et al., 2012).

Thus, for environmental reasons, the biotreament of leather using an enzymatic approach is preferable as it offers several advantages, e.g. easy control, speed and waste reduction, thus being ecofriendly (Shampa Sen et al., 2009).

Alkaline proteases with elastolytic and keratinolytic activity can be used in leather-processing industries.

Alkaline proteases alone account for 20% of the world enzyme market with their predominant use in leather processing and detergent industries (Oberoi et al., 2001; Saeki et al., 2007; Dias et al., 2008; Mukesh Kumar et al., 2012 Agrawal et al., 2012).

Proteases in Food and Pharmaceuticals:
Proteases are widely used in brewing, baking, tenderization of meat, dairy industry, synthesis of aspartame, developing effective therapeutic agents, treatment of wounds (Kalisz, 1988; Cowan, 1996; Kumar and Takagi, 1999; Inhs et al., June 1999; Kumar and Tagaki, 1999; Ramachandran et al., 2004; Alya Sellami-Kamoun et al., 2006; Hamid Mukhtar and Ikram-ul-Haq, 2008; Mayuri Sharma et al., 2019). Proteases are invariably used in tonics, especially for indigestion. Fungal alkaline proteases are also used in food protein modification (Rao et al., 1998; Southan, 2001; Padmavathi, 2013; Charu Lata et al., 2014).

Proteases to be used in the food industry are mainly produced by fungi, whereas the proteases used in leather or bacteria (Hamid Mukhtar and Ikram-ul-Haq, 2008; Noora Barzkar (2020)) mainly produce detergent industries. Alkaline proteases are of special interest as they could be used in manufacture food, such as cheese making baking, preparation of soya hydrolysates, and meat ten-derization pharmaceuticals (Ferrero et al., 1996; Saeki et al., 2007; Dias, et al., 2008 and Mukesh Kumar et al., 2012).

Dairy Industry:
The major application of proteases in the dairy industry is in the manufacture of cheese (Petra Philipps-Wiemann (2018)).

The proteases produced by GRAS (genetically regarded as safe)-cleared microbes such as Mucor michei, Bacillus subtilis, and Endothia parasitica are gradually replacing chymosin in cheesemaking (Rao et al., 1998). In 1988, chymosin produced through recombinant DNA technology was first introduced to cheesemakers for evaluation. Genencor International increased the production of chymosin in Aspergillus niger var. awamori to commercial levels (Xiangyang Liu and Chandrakant Kokare (2017)).

Uses of proteases in Baking Industry:
Endo- and exoproteinases from Aspergillus oryzae are extensively used in production of foods such as syrups, alcohol, fruit juices, brewing, chocolate syrup, baking and meat tenderizing (Luis Carlos Gioia et al., 2017).

Synthesis of Aspartame:
The use of aspartame as a no calorific artificial sweetener has been approved by the Food and Drug Administration. An immobilized preparation of thermolysin from Bacillus thermoprotyolyticus is used for the enzymatic synthesis of aspartame (Charu Lata et al., 2014; Andreas Abelsson (2020)).

Brewing Industry:
The brewing industry is a major user of proteases. In the production of brewing wort Bacillus subtilis protease are used to solublize protein from barley adjuncts, thereby releasing peptides and amino acids which can fulfil the requirement of the nitrogen supply (Agrahari,2011; Rani et al., 2012, Kanupriya et al., 2017).

Medical Usage:
Kudrya and Simonenko (1994) exploited the elastolytic activity of B. Alkaline-fibrinolytic protease has been reported to preferentially degrade fibrin suggesting its future application in thrombolytic therapy and anticancer drugs (Mukherjee and Rai, 2011; Simkhada et al., 2010b; Charu Lata et al., 2014).

Therapeutics:
The use of immobilized alkaline protease from Bacillus subtilis possessing therapeutic properties has been studied for development of soft gel based medicinal formulas, ointment compositions, gauze, non-woven tissues and new bandage materials. Oral administration of proteases from Aspergillus oryzae has been used as a diagnostic aid to correct certain lytic enzyme deficiency syndromes (Rao et al., 1998). Alkaline-fibrinolytic protease has been reported to preferentially degrade fibrin suggesting its future application in thrombolytic therapy and anticancer drugs (Mukherjee and Rai, 2011; Simkhada et al., 2010b; Charu Lata et al., 2014).

Pharmaceutical Industry:
Proteases are useful in the field of medicine also where they have some diagnostic and therapeutic applications (Ward, 1985). Oral administration of proteases from Aspergillus oryzae (Luizym and Nortase) has been used as a digestive aid to correct certain lytic enzyme deficiency syndromes (Rani et al., 2012). An asparginase isolated from E. coli is used to eliminate aspargine from the bloodstream in the various forms of lymphocytic leukemia (Rao et al., 1998; Rani et al., 2012).Proteases are useful in the field of medicine also where they have some diagnostic and therapeutic applications (Ward, 1985).

Proteases in silk degumming in Textile Industry:
Serein, which is about 25%, is conventionally removed from the inner core of fibroin by conducting shrink proofing and twist-setting for the silk yarns, using starch (Kanehisa, 2000).

Photographic Industry-Silver recovery:
Alkaline proteases are used in silver recovery from used X-ray films by decomposing the gelatinous coating on them (Cowan, 1996; Kumar and Takagi, 1999; Anwar and Saleemuddin, 2000; Alya Sellami-Kamoun et al., 2006).

Used X-ray film contains approximately 1.5% to 2.0% (by weight) silver in its gelatin layers. Conventionally, this silver is recovered by burning the films, which causes undesirable environmental pollution (Ray, 2012; Charu Lata et al., 2014), hence the enzymatic hydrolysis of the gelatin layers on the X-ray film enables the recycling of both silver and poly-ester film base (Debette, 1991; Shampa Sen et al., 2009). Alkaline protease from B. subtilis decomposed the gelatin layer within 30 min at 50-60°C and released the silver.

Alkaline Proteases, a tool to clean environment/Waste Management:
Extracellular enzymes have ability to enhance bioremediation because of break down bonds within organic compounds and/or catalyse and transform into less toxic and more biodegradable forms (Vipul Kumar et al., 2019). Alkaline proteases are being able to reduce pathogen counts, solids content, and increase deflocculation in sludge (Passanai, (2020)).

Management of Industrial and House-hold wastes:
Protein-based residues usually represent the most significant potential foul ants within food bioprocess sectors, such as milk- and meat-processing operations. Pretreatment with NaOH, mechanical disintegration, and enzymatic hydrolysis resulted in total solubilization of the feathers. The ended product was a heavy, grayish powder with a very high protein content, which could be used as a feed additive (Mayuri Sharma et al., 2019). Similarly, many other keratinolytic alkaline proteases were used in feed technology for the production of amino acids.

Peptide Synthesis:
Enzymatic peptide synthesis offers several advantages over chemical methods, e.g. reactions can be performed stereo specifically and reactants do not require side-chain protection, increased solubility of non-polar substrates, or shifting thermodynamic equilibria to favor synthesis over hydrolysis (Wuts, Peter and Greene, Theodora (2006) In addition to demonstrating high organic tolerance, alkaline proteases from B.pumilus strain CBS and Streptomyces sp. strain AB1 are potential strong canditates for use in peptide synthesis in low water systems (Jaouadi et al., 2011; Mikawlrawng (2016).

CONCLUSION AND FURTHER APPLICATIONS:

There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS.

Expansion of biotechnology tools offers a constructive position in the development of proteases facilitate their applications to provide a sustainable environment achieved to attain the healthy human life. Acknowledgments: I would like to thank Dr. Suresh Vundavalli for his continuous help and encouragement to complete this article. For the provision of required amenities and facilities of the J.M.J.College, Tenali, Guntur, Andhra Pradesh and India to carry out the research and bring this article to print form.

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